The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist

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Abstract
Nesfatin-1 is an 82 amino acid protein encoded by the nucleobindin2 gene. When injected intracerebroventriculally, nesfatin-1, via a melanocortin ¾ receptor-dependent mechanism, potently decreased both food and water intakes and elevated mean arterial pressure in a dose-related manner. Because nesfatin-1 colocalized with oxytocin in hypothalamus, and because nesfatin-1 had direct depolarizing effects on oxytocin producing neurons in hypothalamic slice preparations, we hypothesized that the actions of nesfatin-1 required the presence of functional oxytocin receptors. We therefore pretreated conscious, unrestrained male rats with the oxytocin receptor antagonist, OVT, before treatment with nesfatin-1. We found that pretreatment with OVT reversed the effects of nesfatin-1 on both food and water intake and on mean arterial pressure, indicating that the central oxytocin system is a downstream mediator of these actions of nesfatin-1. Additionally, we found that OVT reversed the anorexigenic effect of alpha-MSH, suggesting that the central oxytocin system is downstream of the central melanocortin system. Taken together, these data suggest that nesfatin-1 acts through serial neuronal circuit, in which nesfatin-1 activates the central melanocortin system, which in turn acts through the central oxytocin system, leading to an inhibition of food and water intake and an increase in mean arterial pressure.
Introduction

Nesfatin-1 is a recently discovered, 82 amino acid protein derived from the nucleobindin2 (NUCB2) precursor (Oh-I et al., 2006). Nesfatin-1 is produced in several hypothalamic nuclei, such as the paraventricular nucleus (PVN), supraoptic nucleus (SON), arcuate nucleus, and lateral hypothalamic area (LHA) (Oh-I et al., 2006), and in extra-hypothalamic areas as well, including the raphe pallidus, the Edinger-Westphal nucleus, and the nucleus of the solitary tract (NTS) (Foo et al., 2008). Although nesfatin-1 has been shown to colocalize with several well-described peptides, including cocaine and amphetamine-regulated transcript (CART), corticotropin releasing factor (CRF), oxytocin, and vasopressin, nesfatin-1 has not been visualized in axons terminals (Foo et al, 2008). This has led several groups (Foo et al., 2008; Stengel et al., 2009; Yosten and Samson, 2009) to speculate that nesfatin-1 does not signal via a classical axonal mechanism, but rather is released dendritically to act locally in a paracrine or autocrine fashion.

Nesfatin-1 originally was shown to be a potent inhibitor of both food and water intake via a leptin-independent, melanocortin receptor-dependent mechanism (Oh-I et al., 2006; Yosten and Samson, 2009). Nesfatin-1 is likely a physiologically relevant regulator of food intake, as chronic central administration of a morpholino antisense oligonucleotide led to exaggerated food intake and weight gain over missense-injected controls (Oh-I et al., 2006). Additionally, plasma levels of nesfatin-1 were significantly reduced by fasting, and this effect was reversed by refeeding (Stengel et al., 2009). We recently have shown that nesfatin-1 increases mean arterial pressure (MAP) when injected into the lateral cerebroventricle (i.c.v.) (Yosten and Samson, 2009). This effect,
like the effect of nesfatin-1 on food intake, was blocked by pretreatment with the melanocortin ¾ receptor antagonist, SHU9119 (Yosten and Samson, 2009). The increase in MAP induced by nesfatin-1 also was blocked by pretreatment with the non-specific alpha-adrenergic antagonist, phentolamine, suggesting that nesfatin-1 acts through the central melanocortin system to increase sympathetic nervous system activity, leading to an elevation in MAP (Yosten and Samson, 2009). In addition to the central melanocortin system, it appears that a forebrain site of action involving the recruitment of CRF neurons underlies the anorexigenic action of nesfatin-1, when administered into the lateral, but not the fourth, cerebroventricle (Stengel et al., 2009). It is unknown, however, what other neuronal populations may be involved in the expression of the anorexigenic and hypertensive effects of nesfatin-1.

Oxytocin was originally described based on its effects on uterine contractility and mammary tissue (den Hertog et al., 2001; Du Vigneaud et al., 1954). The central oxytocin system, which is comprised of both magnocellular (projecting to posterior pituitary and locally acting via dendritic release) and parvocellular (projecting to brainstem and other brain sites) oxytocin-producing neurons, is important for the expression of maternal, sexual, and feeding behaviors, and the control of cardiovascular function as well (Sabatier et al., 2003b; Michelini et al., 2003). Because nesfatin-1 colocalized with oxytocin in neurons in the PVN (Foo et al., 2008), and because nesfatin-1 had direct depolarizing effects on oxytocin neurons in hypothalamic slice preparations (Price et al., 2008), we hypothesized that the central oxytocin system was a downstream mediator of the anorexigenic, antidipsogenic, and hypertensive activities of nesfatin-1. Indeed, a recent report (Maejima et al., 2009) demonstrated that the inhibitory effect of
nesfatin-1 on food intake was dependent on central oxytocin receptors. Here we confirm the findings of Maejima et al. on food intake, and add that the effects of nesfatin-1 on water intake and on MAP could be blocked by pretreatment with the oxytocin receptor antagonist, ornithine vasotocin (OVT). We also add that the anorexigenic action of alpha-melanocyte stimulating hormone (alpha-MSH) was reversed by pretreatment with OVT, suggesting that nesfatin-1 acts through a serial neuronal circuit, in which nesfatin-1 activates the central melanocortin system, which in turn acts through the central oxytocin system to increase mean arterial pressure and inhibit food and water intake.

Materials and Methods

All procedures and protocols have been approved by the Saint Louis University Animal Use and Care Committee (protocol number 2041). Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed under controlled conditions (23-25°C, lights on 0600-1800hr) with free access to food and water. Rats (225-250g; approximately 7-8 weeks of age) were anesthetized with a mixture of ketamine (60mg/ml; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (8mg/ml; TranquiVed, VedCo, Saint Joseph, MO) at a dose of 0.1 ml per 100 g body weight, injected intra-peritoneally, as previously described (Yosten and Samson, 2009). Buprenorphine (0.05 mg/kg body weight, injected subcutaneously) was administered post-anesthesia for analgesia. Fluid replacement included a subcutaneous injection of sterile saline (0.9% NaCl) to balance anticipated fluid loss (3:1). A stainless steel cannula (23 mm, 17 gauge) was implanted into the right lateral cerebroventricle (i.c.v.) using a stereotaxic device (coordinates
relative to the interaural line: A: +6.2, L:+7.4, H:-0.9) and immobilized using dental cement. Rats were then housed singly and observed for at least 4 days following surgery to ensure recovery of pre-surgery weight. Placement and patency of the i.c.v. cannula was confirmed by the dipsogenic effect of angiotensin II (50 picomoles i.c.v.).

For feeding studies, rats bearing an i.c.v. cannula were habituated to metabolic cages (Nalgene) for 3 days. Food and water intakes and body weights were monitored daily to ensure health. On the day of the experiment, food and water were removed from the cages at 1650, and rats were pretreated i.c.v. with 2 microliters saline vehicle or 10 ug [D-(CH2)5,Tyr(me)2,Orn8]-vasotocin (OVT) (Arletti et al., 1989). 1-10 minutes later, rats were then injected with saline, 60 pmole nesfatin-1, an anorexigenic dose of oxytocin (0.5 micrograms) (Olson et al., 1991), or an anorexigenic dose of alpha-MSH (1.0 nmole) (Rossi et al., 1998). Food and water bottles were replaced 10 minutes later, and food and water intakes were monitored at 30 minute intervals for one hour during the light phase (1700-1800) and three hours during the dark phase (1800-2100), and again 24 hours later. Experiments are conducted during this time frame so as to coincide with the natural light-entrained feeding cycle of our rat colony.

For cardiovascular experiments, an additional cannula (PE-50) was implanted into the left carotid artery of rats minimally five days after implantation of the i.c.v. cannulae, and exteriorized between the shoulder blades as previously described (Yosten and Samson, 2009). The cannula was filled with heparinized saline (200 U/ml in 0.9% NaCl). The next day (during lights on, between 0600 and 1800), rats were placed in a quiet room, and after minimally two hours habituation the carotid cannula was connected to a pressure transducer (DigiMed Blood Pressure Analyzer, Micro-Med, Louisville,
Baseline MAP and heart rate (HR) then were recorded at 1 minute intervals for at least 30 minutes. Rats were pretreated with either 2 microliters saline vehicle or vehicle containing 10 ug OVT (i.c.v.). Ten minutes later, rats were treated with either saline vehicle or 180 pmole nesfatin-1 (i.c.v.) and MAP and HR were recorded for at least 15 minutes at 1 minute intervals. Data are represented as change from pre-injection baseline, which was determined by averaging the MAP or HR values for 5 minutes before injection of nesfatin-1 or saline vehicle.

To determine plasma oxytocin levels, rats that were habituated to a quiet room were injected i.c.v. with either saline vehicle or 180 pmole nesfatin-1. 10 minutes later, rats were sacrificed by rapid decapitation and trunk bloods were collected. Plasma oxytocin levels were determined by radioimmunoassay as previously described (Samson et al., 2004). The lower limit of sensitivity was defined as minimally 95% of total binding (2 pg/ml plasma).

Feeding data were analyzed using ANOVA with Scheffe’s multiple comparison. Cardiovascular data were analyzed using a non-parametric test (Mann-Whitney U), as MAP/HR data were transformed to represent change from pre-injection baseline because of the natural variation of resting MAP/HR between animals. Radioimmunoassay data were analyzed using a t test. All peptides were purchased from Phoenix Pharmaceuticals (Burlingame, CA). 60 pmole and 180 pmole nesfatin-1 were used for feeding experiments and cardiovascular experiments, respectively, because these were the most effective doses in each experimental paradigm (Yosten and Samson, 2009). The dose of OVT used in this study was determined based on the dose previously reported in the literature (Arletti et al., 1989).
Results

Oxytocin inhibited both food and water intake when injected into the lateral cerebroventricle (Figure 1A/B). Although food intake was decreased at all time points compared to saline-injected controls, this effect was most clear during later time points, when the effect attained significance. The effect of oxytocin on water intake was less robust than the effect on food intake, but did attain significance during the last 3 time intervals. Pretreatment with the oxytocin receptor antagonist, OVT, reversed oxytocin-induced anorexia and adipsia.

Central administration of 60 pmole nesfatin-1 significantly inhibited cumulative food intake as previously reported (Oh-I et al., 2006; Yosten and Samson, 2009; Stengel et al., 2009). This decrease attained significance at the first three sampling periods when compared to cumulative food intakes in animals administered saline vehicle alone or the oxytocin antagonist and saline (nesfatin-1 induced an approximately 55% decrease in food intake compared to controls at 1730) (Figure 2A). Pretreatment with OVT reversed the inhibitory action of nesfatin-1, but did not affect food or water intake when injected alone. This reversal attained significance at the 30 and 60 minute sampling intervals. Cumulative food intakes in animals administered nesfatin-1 after OVT at no time point differed significantly from intakes observed in saline vehicle controls or animals administered OVT and saline. When the data were analyzed by ANOVA, no significant differences in cumulative food intakes among treatment groups were observed at any subsequent (following 1900) sampling intervals (data not shown).
Central administration of 60 pmole nesfatin-1 significantly inhibited cumulative water intake as previously reported (Yosten and Samson, 2009). This decrease attained significance at the first three sampling periods when compared to cumulative water intakes in animals administered saline vehicle alone or the oxytocin antagonist and saline (nesfatin-1 induced an approximately 82% decrease in water intake compared to controls at 1730) (Figure 2B) and in the fourth interval (1900 hr) compared to water consumed in OVT and saline treated animals. Cumulative water intakes in animals administered nesfatin-1 after OVT at no time point differed significantly from intakes observed in saline vehicle controls or animals administered OVT and saline. However, a significant reversal of the inhibitory effect of nesfatin-1 on water drinking was observed following OVT pretreatment at 90 minutes. While more water was consumed by animals treated with OVT and nesfatin-1 compared to those treated with saline and nesfatin-1 at all sampling times, this attained significance only at the 90 minute sampling interval. Increased water consumption was observed in the nesfatin-1 treated animals following the fourth sampling interval (after 1900 hr) such that no significant differences in cumulative water consumption were observed compared to controls thereafter (data not shown).

Because the anorexigenic and antidipsogenic effects of nesfatin-1 were completely reversed by pretreatment with a melanocortin 3 receptor antagonist (Oh-I et al., 2006; Yosten and Samson, 2009) and an oxytocin receptor antagonist (Figure 2A/B; Maejima et al., 2009), we hypothesized that nesfatin-1 acts through a serial neuronal circuit to exert its activities. Since melanocortin agonists previously were shown to increase oxytocin release (Sabatier et al., 2003a), we reasoned that the central oxytocin
system acts as a downstream mediator of the central melanocortin system. To test this hypothesis, we pretreated rats with either saline or OVT i.c.v. prior to central administration of saline or an anorexigenic dose of alpha-MSH (Rossi et al., 1998). Animals that received saline and alpha-MSH consumed significantly less food and water than animals that were injected with saline alone (Figure 3A/B). However, the effect of alpha-MSH on food intake was reversed by pretreatment with OVT. OVT also reversed the effect of alpha-MSH on water intake, although this effect did not attain significance until 1900 hours.

Because nesfatin-1 acts through the central melanocortin system to affect both food and water intake and MAP (Oh-I et al., 2006; Yosten and Samson, 2009), we sought to determine if the central oxytocin system was a point of convergence or divergence for the different effects of the protein (i.e. appetitive versus autonomic). We therefore tested the ability of OVT to block the hypertensive action of nesfatin-1.

In conscious, freely moving rats, 180 pmole nesfatin-1 led to significant increases in MAP (Figure 4A), as previously reported (Yosten and Samson, 2009). Pretreatment with 10 ug OVT did not affect resting MAP (Table 1), nor did OVT/saline-treated rats exhibit any alterations from pre-injection baseline. However, pre-treatment with OVT completely abolished the stimulatory effect of nesfatin-1 on MAP. No significant increases in HR were observed in any of the treatment groups (Figure 4B). HR was only significantly decreased in saline/nesfatin-1-treated rats at one time point, 13 minutes post-injection (pre-injection HR baselines: saline/saline=388±10; saline/nesfatin-1=387±11; OVT/saline=408±14; OVT/nesfatin-1=388±19).
The effects of nesfatin-1 were shown previously to be dependent on the central melanocortin system (Oh-I et al., 2006; Yosten and Samson, 2009). Because melanocortin agonists were shown to stimulate the release of oxytocin from dendrites but inhibit axonal release of oxytocin (Sabatier et al., 2003a), we sought to determine if central injection of nesfatin-1 would alter plasma oxytocin levels. Rats were therefore treated with either saline or 180 picomole nesfatin-1 i.c.v. Ten minutes later, trunk bloods were collected and plasma oxytocin levels were determined by radioimmunoassay. Nesfatin-1 did not significantly alter plasma oxytocin levels [Saline = 10.4 ± 0.6 (n=14); nesfatin-1 = 9.8 ± 1.0 (n=14)].

Discussion

Oxytocin has been shown to act in brain to inhibit food intake (Olson et al., 1991) and alter cardiovascular function (Michelini et al., 2003; Wsol et al., 2008), and is a potential downstream mediator of the cardiovascular effects of substance P (Maier et al., 1998) and neuropeptide FF (Jhamandas and MacTavish, 2003). Oxytocin colocalizes with nesfatin-1 in the PVN (Foo et al., 2008), and nesfatin-1 has direct depolarizing effects on oxytocin neurons in hypothalamic slice preparations (Price et al., 2008). These findings suggest that nesfatin-1 could interact with central oxytocin receptors to exert its anorexigenic and hypertensive effects.

In these studies, we found that the actions of nesfatin-1 on food and water intake and MAP could be blocked by pretreatment with the oxytocin receptor antagonist, OVT, suggesting that functional oxytocin receptors are required to mediate the actions of nesfatin-1. Although it has been reported previously (Fitts et al., 2003) that OVT alone
led to an exaggeration of water intake, this effect was not observed in our experiments. This is likely due to strain differences, as the studies by Fitts and colleagues utilized Long-Evans rats, while all of our experiments are conducted using Sprague-Dawley rats. In our experiments, OVT did not alter food intake. This is in accordance with previous a report (Blevins et al., 2004) which demonstrated that OVT does not lead to exaggerated food intake unless injected into the fourth ventricle (our injections are made into the lateral ventricle). We previously have reported that the activities of nesfatin-1 also require the presence of functional melanocortin receptors (Yosten and Samson, 2009).

While it is possible that the central melanocortin system and the central oxytocin system operate in parallel to simultaneously exert the effects of nesfatin-1, these two neuronal circuits may also act in series. Melanocortin agonists and oxytocin exerted similar actions on a variety of physiological functions when injected into brain, including the initiation of the yawning-stretching reflex and sexual behaviors (Sabatier et al., 2003b). Central administrations of oxytocin (Michelini et al., 2003; Wsol et al., 2008; Olson et al., 1991) and melanocortin agonists (Cone, 2005) led to a potent inhibition of food intake and altered cardiovascular function. These similarities suggest a circuit in series.

Several lines of evidence support the hypothesis that oxytocin is a downstream mediator of the central melanocortin system. Intracerebroventricular administration of the melanocortin ¾ receptor agonist, alpha-melanocyte stimulating hormone (alpha-MSH) led to c-Fos accumulation in oxytocin-producing neurons (Caquineau et al., 2006). Pretreatment with OVT reversed the anorexigenic effect of leptin (Blevins et al., 2004), a peptide that is dependent on the central melanocortin system to exert its activities (Dunbar and Lu, 1999). Here we also show that the anorexigenic effect of alpha-MSH is
reversed by pretreatment with OVT (see Figure 3). Additionally, alpha-MSH was shown to increase the dendritic release of oxytocin in hypothalamus, but to inhibit the release of oxytocin from axon terminals in the posterior pituitary gland leading to a decrease in plasma OT levels (Sabatier et al., 2003a). Nesfatin-1 may indeed increase dendritic release of oxytocin, as a recent report (Maejima et al., 2009) indicated that nesfatin-1 led to an increase in oxytocin secretion within the PVN and evidence from our laboratory indicates that nesfatin-1 does not affect basal plasma levels of oxytocin in conscious male rats. In our experiments, we did not observe a decrease in plasma oxytocin levels, unlike the decrease in oxytocin reported by Sabatier and colleagues (2003a) after treatment with melanocortin agonists. The difference is likely due to differences in model systems.

Sabatier et al. conducted their studies using female rats that were injected with hypertonic saline to increase basal oxytocin levels, while all of our experiments were performed using male rats, in which basal oxytocin levels were extremely low. Nesfatin-1 may also increase axonal release of oxytocin from parvocellular neurons, as nesfatin-1 led to an increase in c-Fos expression in parvocellular PVN neurons (Maejima et al., 2009). A recent report (Tolson et al., 2010) also provided evidence that conditional Sim1 knockout mice are hyperphagic and obese, and exhibit a marked reduction in melanocortin 4 receptor and oxytocin expression in the hypothalamus. Their data support our hypothesis of an action of nesfatin-1 on POMC neurons in ARC, resulting in the activation of OT neurons in PVN by alpha-MSH derived from those ARC POMC neurons.

Stengel and colleagues have reported that nesfatin-1 injected into the lateral cerebroventricle reduced food intake, and this effect was reversed with the CRF2 receptor antagonist, astressin2-B (Stengel et al., 2009) suggesting an action of nesfatin-1 on CRF
neurons in forebrain. CRF is another potential downstream mediator of the central melanocortin system, as the anorexigenic effect of the melanocortin agonist MTII was abolished by a CRF receptor antagonist (Lu et al., 2003), and Briscoe and coworkers (Briscoe et al., 2009) demonstrated that, like nesfatin-1 (Yosten and Samson, 2009) and oxytocin (Richard et al., 1991; Michelini et al, 2003; Bernatova et al., 2004; Wsol et al., 2008), central administration of CRF resulted in an increase in mean arterial pressure. Thus, interactions with not only the melanocortin and oxytocin pathways, but also with forebrain CRF neurons may underlie the anorexigenic and the sympathostimulatory actions of nesfatin-1.

After the completion of our studies described here, while preparing this manuscript for publication, Maejima et al. reported that the effect of nesfatin-1 on food intake was blocked by pretreatment with the oxytocin receptor antagonist, H4928 (Maejima et al., 2009). Those authors also provided evidence that pretreatment with the melanocortin 3/4 receptor antagonist, SHU9119, reversed the effect of oxytocin on food intake, indicating that nesfatin-1 may act through the central oxytocin system to activate the central melanocortin system, leading to an inhibition of food intake. However, data from other groups (Sabatier et al, 2003a; Caquineau et al., 2006; Blevins et al., 2004), as mentioned above, and data from the studies described here, suggest that the order of activation is reversed. Our data are in agreement with those of Maejima et al., supporting a role for OT neurons in the anorexigenic action of nesfatin-1. Here we add that an oxytocin receptor antagonist, OVT, also abrogated the antidipsogenic and hypertensive effects of nesfatin-1. These two additional, novel observations further support the hypothesis that the CNS actions of nesfatin-1 are dependent upon not only the activation
of the central melanocortin system (Oh-I et al., 2006; Yosten and Samson, 2009), but also on the activation of OT neurons that project to CNS sites known to be important in the control of appetite and autonomic regulation.

**Perspectives**

Since nesfatin-1 has not been visualized in axon terminals (Foo et al., 2008), and because the protein was localized to secretory vesicles in perikarya (Maejima et al., 2009), it is tempting to hypothesize that nesfatin-1 is a locally-acting, modulatory protein. Target sites of nesfatin-1 action may include areas where the protein is produced, such as the hypothalamus and the nucleus of the solitary tract. Nesfatin-1 likely exerts the same activities in both of these (and in other) brain sites, since nesfatin-1 inhibited food intake when injected into the lateral ventricle, the third ventricle, the fourth ventricle, or cisterna magna (Yosten and Samson, 2009; Oh-I et al., 2006; Stengel et al., 2009). Interestingly, Skibicka and Grill (2009) recently have shown that melanocortin agonists increased heart rate and thermogenesis and inhibited food intake regardless of the injection site (2 forebrain sites and 3 hindbrain sites). Which neuronal system(s) is (are) the primary target of nesfatin-1 remains unknown. Nesfatin-1 colocalized with both oxytocin and POMC in brain (Foo et al., 2008), and the actions of nesfatin-1 were blocked by pretreatment with both SHU9119 (Oh-I et al., 2006; Yosten and Samson, 2009) and oxytocin receptor antagonists (Maejima et al., 2009; this manuscript). Further studies are required to determine if nesfatin-1 is acting through these two systems simultaneously, or if there exists a hierarchy of neural networks organized to mediate the effects of nesfatin-1 on both appetite and autonomic function that has as an anatomic basis a shared series element, requiring activation initially of POMC and then OT neurons, or vice-versa, both
potentially recruiting CRF neurons in hypothalamus (Stengel et al., 2009). Behavioral actions of nesfatin-1 (Merali et al., 2008; Yosten and Samson, 2009) may require activation of similar circuitry as well.
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References

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supraoptic nuclei of the rat hypothalamus coexpress oxytocin and vasopressin and are activated by refeeding. *Endocrinology* 149: 1295-301, 2008.


27. **Samson WK, Baker JR, Samson CK, Samson HW, Taylor MM.** Central


Figure Legends

**Figure 1:** The anorexigenic and antidipsogenic effects of oxytocin are blocked by pretreatment with OVT. Rats were pretreated with either saline vehicle or vehicle containing 10 ug OVT i.c.v., and then were administered either saline or 0.5 ug oxytocin i.c.v. While oxytocin significantly inhibited both food (panel a) and water (panel b) intake, these effects were reversed by pretreatment with OVT. Data were analyzed using ANOVA with Scheffe’s multiple comparisons (*p<0.05, **p<0.01, ***p<0.001, versus saline-injected controls).

**Figure 2:** The anorexigenic and antidipsogenic effects of nesfatin-1 are blocked by pretreatment with OVT. Rats were pretreated i.c.v. with either saline vehicle or vehicle containing 10 ug OVT, then treated with either saline vehicle or 60 pmole nesfatin-1. While nesfatin-1 significantly reduced both food (panel a) and water (panel b) intake, these effects were reversed by pretreatment with OVT. Data were analyzed using ANOVA with Scheffe’s multiple comparisons (*p<0.05, **p<0.01, ***p<0.001, versus saline-injected controls).

**Figure 3:** The anorexigenic and antidipsogenic effects of alpha-MSH are blocked by pretreatment with OVT. Rats were pretreated with either saline vehicle or vehicle containing 10 ug OVT i.c.v., then administered either saline or an anorexigenic dose (1.0 nmole) of alpha-MSH i.c.v. While alpha-MSH significantly inhibited both food (panel a) and water (panel b) intake, these effects were reversed by pretreatment with OVT. Data were analyzed using ANOVA with Scheffe’s multiple comparisons (*p<0.05, **p<0.01, ***p<0.001, versus saline-injected controls).

**Figure 4:** Nesfatin-1-induced elevation in MAP is blocked by pretreatment with OVT. Rats bearing i.c.v. and carotid cannulae were pretreated with 10 ug OVT or saline vehicle i.c.v. 10 minutes later, rats were treated with either saline vehicle or 180 pmole nesfatin-1 (Time 0). While nesfatin-1 led to significant increases in MAP (panel a), this effect was blocked by pretreatment with OVT. None of the treatment groups exhibited any significant increases in HR (panel b). Data are represented as change from pre-injection baseline (average 5 minutes before i.c.v. injection). Data were analyzed using a Mann-Whitney U test (*p<0.05, **p<0.01, ***p<0.001, versus saline-injected controls).

**Table 1:** Neither OVT nor saline pretreatment altered baseline MAP. Data are presented as average MAP for 5 minutes before or after pretreatment with either 10 ug OVT or saline vehicle i.c.v.
Cumulative Food Intake (g/100 g body weight, Mean, SEM)

- **Vehicle/Vehicle (n=8)**
- **Vehicle/1.0 nmole α-MSH (n=7)**
- **10 μg OVT/1.0 nmole α-MSH (n=6)**

**Time of Day (Hours)**
- 1730
- 1800
- 1830
- 1900
- 1930
- 2000
- 2030
- 2100

*Significance levels: *
- *p < 0.05
- **p < 0.01
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